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Cryptophyta as major bacterivores in freshwater summer plankton

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Abstract: Small bacterivorous eukaryotes play a cardinal role in aquatic food webs and their taxonomic classification is currently a hot topic in aquatic microbial ecology. Despite increasing interest in their diversity, core questions regarding predator–prey specificity remain largely unanswered, e.g., which heterotrophic nanoflagellates (HNFs) are the main bacterivores in freshwaters and which prokaryotes support the growth of small HNFs. To answer these questions, we fed natural communities of HNFs from Římov reservoir (Czech Republic) with five different bacterial strains of the ubiquitous betaproteobacterial genera *Polynucleobacter* and *Limnohabitans*. We combined amplicon sequencing and catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) targeting eukaryotic 18 S rRNA genes to track specific responses of the natural HNF community to prey amendments. While amplicon sequencing provided valuable qualitative data and a basis for designing specific probes, the number of reads was insufficient to accurately quantify certain eukaryotic groups. We also applied a double-hybridization technique that allows simultaneous phylogenetic identification of both predator and prey. Our results show that community composition of HNFs is strongly dependent upon prey type. Surprisingly, Cryptophyta were the most abundant bacterivores, although this phylum has been so far assumed to be mainly autotrophic. Moreover, the growth of a small lineage of Cryptophyta (CRY1 clade) was strongly stimulated by one *Limnohabitans* strain in our experiment. Thus, our study is the first report that colorless Cryptophyta are major bacterivores in summer plankton samples and can play a key role in the carbon transfer from prokaryotes to higher trophic levels.

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Supplemental Material

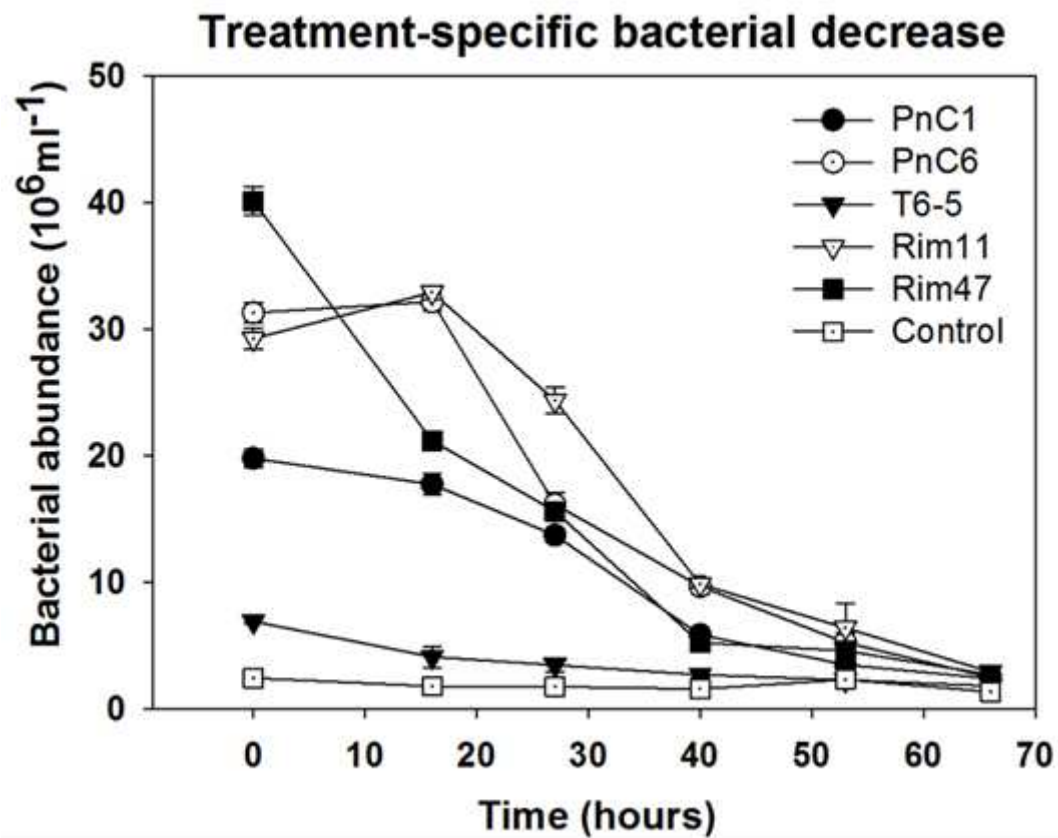


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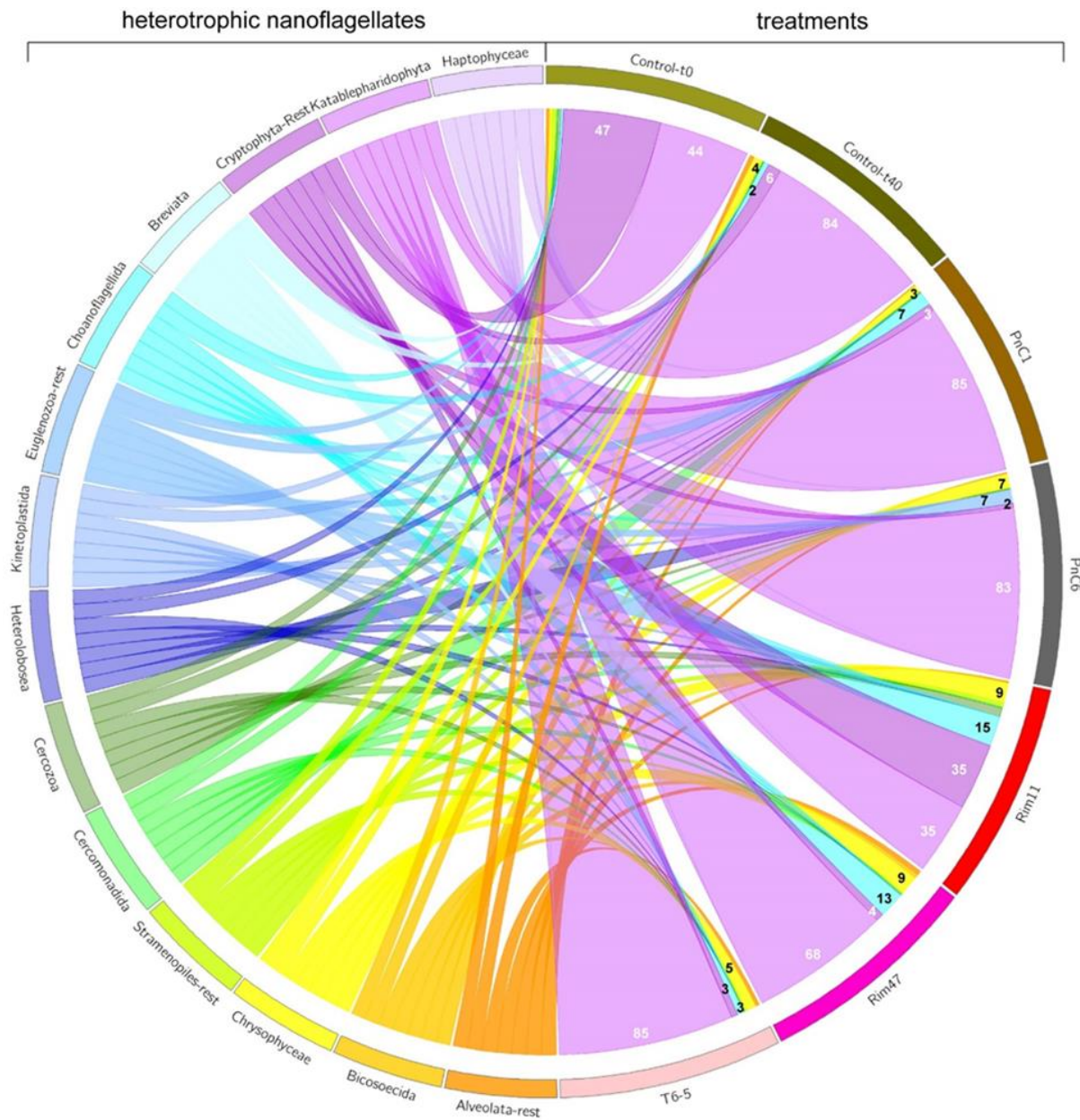
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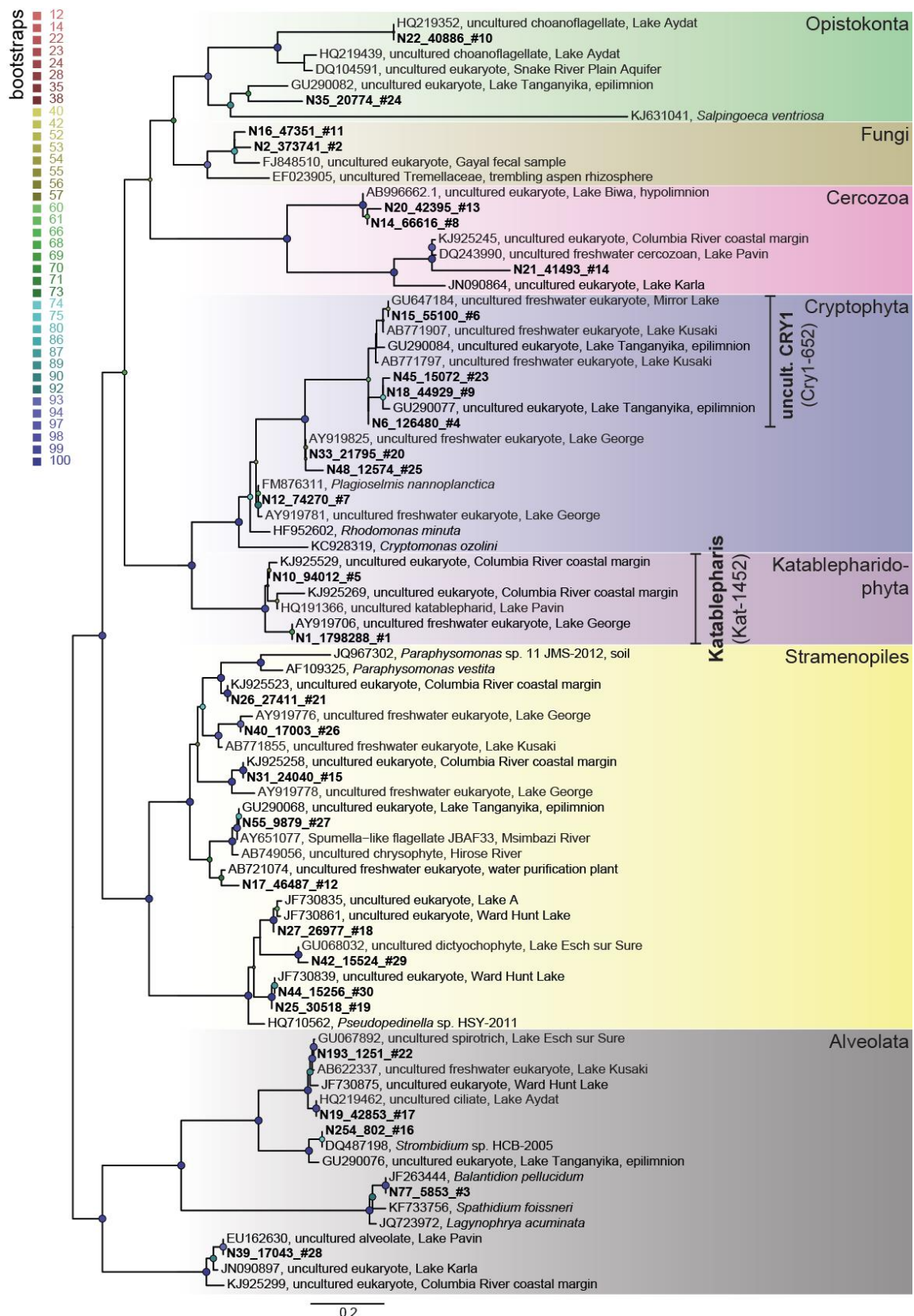
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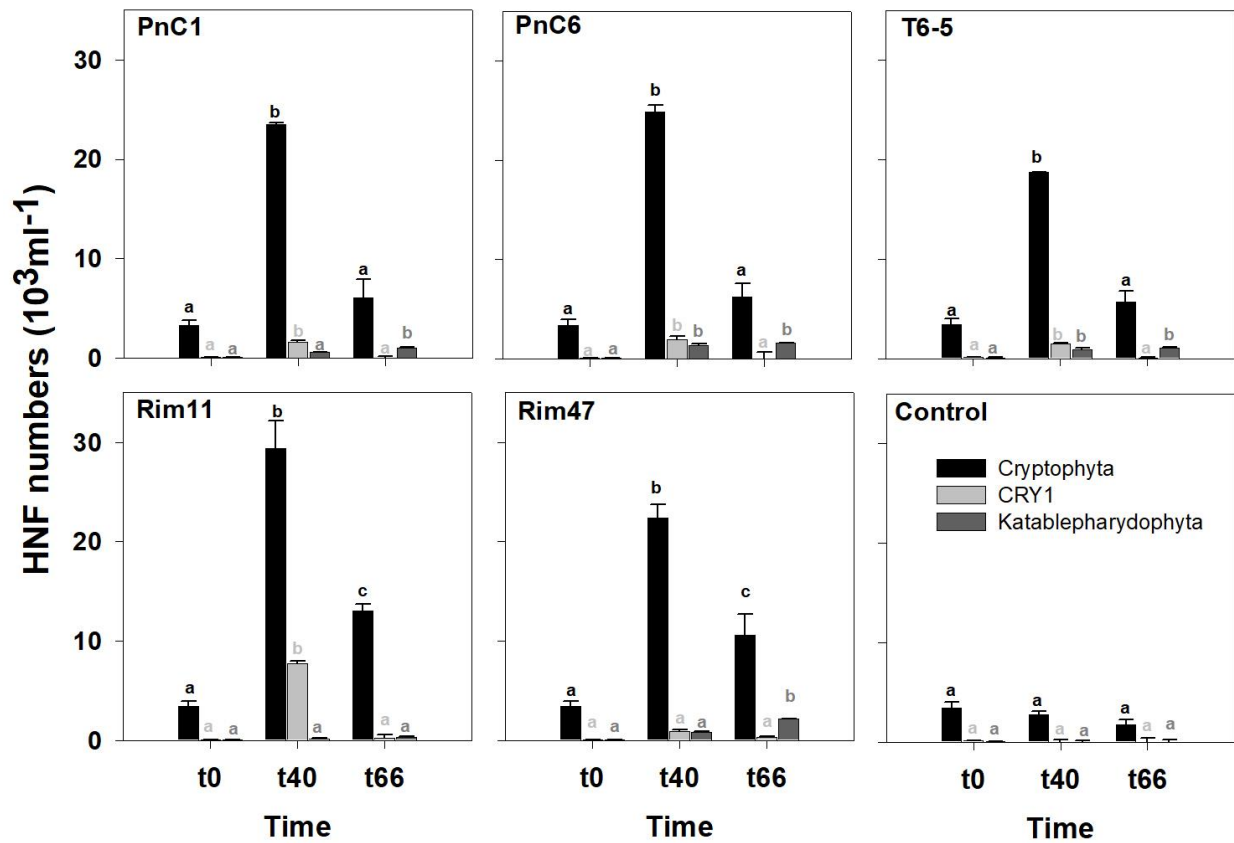
Supplementary Figure 1. Bacterial numbers in experimental treatments amended with bacterial strains (PnC1, PnC6, T6-5, Rim11 and Rim47) compared to the control where no bacterial strains were added at different times of the experiment. Values are means of triplicates. Error bars depict standard deviations.



Supplementary Figure 2. Percentages of reads affiliated with particular taxonomical groups of protists in different treatments and time points. The left half of the circus plot shows the occurring flagellate groups; the right half shows the treatments. Control t0 represents the starting community from the reservoir. Control t40 represents the control treatment after 40 hours of experiment, PnC1, PnC6, T6-5, Rim47, and Rim11 show the protistan community in the bacterial prey-amended treatments after 40 hours of experiment. Values are means of triplicates expressed as percentages.



Supplementary Figure 3. Bootstrapped maximum likelihood tree of eukaryotic 18S rRNA genes including representative sequences of the 30 most abundant OTUs from the amplicon dataset (marked in bold; OTU rank is indicated by #). Sequences targeted by the newly designed probes Cry1-652 and Kat-1452 are shown in brackets. Bootstrap values are indicated by differentially colored circles on nodes, the scale bar at the bottom applies to 20% sequence divergence.



Supplementary Figure 4. Absolute abundances of HNF cells hybridized with probes targeting all Cryptophyta, lineage CRY1, and all Katablepharidophyta at three different time points: t₀, beginning of experiment, representing the starting community from the reservoir; t₄₀ and t₆₀ represent percentage after 40 and 60 hours of experiment. Different letters above the columns indicate significant differences between different times of the experiment within one treatment targeted with one probe (post hoc Tukey test). Values are means of triplicates.